

REMARKS

Claims 1-38 are pending in this application. All the pending claims have been rejected for obviousness under 35 U.S.C. § 103(a).

Applicants and the undersigned conducted a personal interview with Examiners Katcheves and Ketter on March 27, 2006. Applicants wish to thank Examiners Katcheves and Ketter for the courtesies extended at the interview. At the interview all the claims were discussed with respect to the cited art for the outstanding Section 103 obviousness rejection. The presently claimed invention and that of the parent patent were also discussed.

It was pointed out at the interview that the differences between Claim 3 and the Henry et al. reference are that Henry et al.'s methods result in integration of Amp<sup>r</sup> into the host yeast without any teaching or suggestion that it would even be desirable to avoid Amp<sup>r</sup> or any other expressed bacterial sequences. Also none of the yeast cells resulting from Henry et al. are prototrophic and all the insertions of the INO1 gene are at the URA3 locus in the host yeast genome.

The Hinchliffe et al., Hoffman et al., and Buxton references were also discussed regarding the relevance of their teachings about the plasmids they employ and any teachings regarding avoidance of bacterial DNA sequences. Applicants argued that Hinchliffe et al., Hoffman et al., and Buxton all teach away from the present invention because they only employ

2µm plasmids which cannot integrate into a host yeast genome and thus always remain extrachromosomal. Therefore teachings in those references regarding avoidance of bacterial sequences are irrelevant because none of the references teach methods which can result in stable integration into the genome of a host yeast cell to yield the claimed cells. The claim language "expressed bacterial sequence" was also discussed as meaning that the bacterial ORI DNA carried on a yeast integration plasmid is not transcribed and no message is ever produced from that sequence. It was suggested to Applicants that further arguments be set forth explaining the invention and the teachings of the prior art.

Applicants respectfully request that the Examiner reconsider the patentability of the inventions of the claims.

#### The Inventions

The inventions of the present application are directed to diploid or haploid yeast cells of the genus Saccharomyces produced by the methods of the parent application, now U.S. Patent 6,645,767. The motivation behind applicants's invention is that bacterial drug resistant genes are never used at any stage in making the yeast integration plasmids claimed in the parent application. Applicants avoid using expressed bacterial sequences by instead using a yeast selection gene marker (preferably yeast LEU2) in the construction of yeast integration plasmids which allows for the replication of the plasmid in a bacterial plasmid amplification host (preferably auxotrophic for

LEU2) and also allows for selection of the transformed bacterial host colonies containing the integration plasmid.

One or a suite of yeast integration plasmids preferably carrying LEU2 as the selection gene marker are used. There is no use at all of any expressed bacterial sequence such as Amp<sup>r</sup> in the yeast integration plasmids. The yeast integration plasmids also carry 1) a particular gene of interest (such as INO1) for one or multiple insertions into host yeast or 2) a group of genes of interest that code for enzymes that make up either a step of a desired metabolic pathway or an entire metabolic pathway. The particular genes of interest are inserted into one or more predetermined mutated homologous loci ("target gene mutations") of the yeast host because the yeast integration plasmids additionally carry targeting gene markers which direct the insertion of the plasmid by homologous recombination into the target gene mutations in the yeast host. The targeting gene markers are chosen to complement or rescue the auxotrophies that result from the target gene mutations in the host genome.

Since the yeast integration plasmids never carried any expressed bacterial DNA sequences to begin with, none can possibly be integrated into the host genome. One also knows exactly where in the host yeast genome each gene of interest is stably integrated.

The methods of the parent application provide the means to stably integrate seven (7) additional genes into a haploid host and fourteen (14) additional genes of interest into

a diploid host. The claimed cells can also be prototrophic. Since the yeast integration plasmids never contained any expressed bacterial sequence, there is no possibility of such sequence integrating into the host yeast genome. These resulting cells are the subject of the claims of the instant application.

In sum the present claims are directed to yeast cells into which controlled amounts of genes of interest are stably integrated into predetermined loci of the host yeast genome while totally avoiding use of expressed bacterial sequences.

#### The Section 103(a) Rejection

Claims 1-38 were rejected under 35 U.S.C. §103(a) for obviousness over Hinchliffe et al. in view of Hoffman et al., Buxton, and Henry et al. for the reasons set forth in the Office Action of February 23, 2005 and additional grounds set forth in the final Office Action. Applicants respectfully disagree with the factual and legal bases for the obviousness rejection. None of these references individually or collectively teach or suggest the claimed yeast cells of the present invention.

It is respectfully noted that the present claims are not just directed to diploid cells; Claims 10-17 and 30-34 are directed to haploid yeast cells.

Applicants respectfully submit that the teachings of Hinchliffe et al. are irrelevant to the present invention. Hinchliffe et al. teach away from using integration plasmids at Col. 2, lines 6-7 ("In general [integrative] yeast

transformation is relatively inefficient ..."). Column 3, lines 59-65 of Hinchliffe et al. cited by the Examiner previously is irrelevant for several reasons. First, the present invention is not directed to "a genetically modified yeast which is capable of expressing a heterologous gene to produce high levels of a commercially important peptide." (Emphasis added). Applicants' genetically modified yeasts are used to produce inositol and insitol-containing phospholipids. The INO1 gene is not being claimed, but is employed as the "gene of interest" for the production of inositol and inositol-containing phospholipids. However, Hinchliffe et al. concluded they favor use of 2µm-based vectors as "the vector of choice." (Col. 3, lines 63-65).

Hinchliffe et al. also perhaps both confuse and teach away at Col. 4, lines 36-49 where they incorrectly use "integration vector" to describe a 2µm plasmid. Whatever plasmid remains after the transformation is however still a 2µm-based plasmid that never integrates into the genome of the yeast host. Hinchliffe et al. goes on to teach "a modified 2µm plasmid" with "no bacterial DNA" (Col. 5, lines 1-9). Such teaching is however irrelevant and fails to anticipate or render the present claims obvious since the 2µm plasmid can never integrate into the host chromosome and therefore the fact that the plasmid does not contain bacterial DNA, is of no moment and utterly irrelevant to the present claimed cells which require chromosomal integration as a claim element. There is no teaching or suggestion in Hinchliffe et al. as to the desirability of how to bridge the huge conceptual gap from a 2µm

episomal plasmid to an integration plasmid as a construction tool.

It is noted that Hinchliffe et al. at Col. 5, lines 46-55 use the term "stably integrate" with reference to the Met-HSA gene to mean "subclone," "insert," or "to carry into" the 2 $\mu$ m "disintegration" plasmid, not to stably integrate the Met-HSA gene into the host cell's chromosome. Hinchliffe et al. at Col 6., line 26 - Col. 7, line 45 only deal with 2 $\mu$ m plasmids and the described FLP recombinations to delete bacterial sequences only work on 2 $\mu$ m plasmids. There is nothing in Hinchliffe et al. which bridges the concepts of chromosomal integration with how to avoid using bacterial sequences.

Hinchliffe et al. also teach that in brewing yeast (Col. 4, lines 22-35) "the recombinant genes present in yeast should, so far as possible, be restricted to the 'gene of interest' and adjacent yeast regulatory genes . . . . [I]t is desirable that the genetically modified yeast should not possess extraneous DNA sequences such as those which are derived from the bacterial portion of the recombinant plasmid." Hinchliffe et al. continue at Col. 5, lines 25-45 to teach the virtues of "the 2 $\mu$ m based disintegration vector of the present invention" in the absence of extraneous bacterial plasmid DNA sequences.

The problem with the foregoing teachings is that the 2 $\mu$ m based disintegration vectors of Hinchliffe et al. can never be used to provide functional copies of the INO1 gene, or any other gene of interest for that matter, which are inserted into target gene mutation loci of a host yeast and stably integrated.

2 $\mu$ m plasmids are extrachromosomal and can never be used to integrate into the host yeast's chromosome. Thus the discussion of the absence of extraneous bacterial plasmid DNA sequences is strictly limited to material (2 $\mu$ m plasmids) that can never integrate into the host genome to arrive at the claimed cells which require such integration as a claim element.

The further discussion of Hinchliffe et al.'s teachings of a yeast plasmid with selection gene markers such as LEU2, etc., a 2 $\mu$ m origin of replication, and a gene of interest which upon transformation into yeast undergoes FLP-mediated recombination to delete any bacterial DNA does not "anticipate" the instant claims. The elements of the claims are not taught in a way that could ever yield the claimed cells. It is respectfully submitted that it is not true that "Absent evidence to the contrary and given that the cited art does teach the elements of the claims, the cells would inherently be capable of insertion of the gene. Indeed, Hinchliffe et al. teach FLP recombination which shows that the cells are capable of insertion." It cannot be emphasized enough that 2 $\mu$ m plasmids never, ever integrate into the host chromosome and thus are "inherently incapable" of insertion of a gene of interest. FLP recombination furthermore cannot be carried out in chromosomes and only can be carried out in extrachomosomal plasmids. Unfortunately, the only plasmids taught by Hinchliffe et al. are 2 $\mu$ m which are incapable of insertion into the host yeast chromosome. FLP recombination can only be carried out on extrachromosomal plasmids. As discussed in the interview

applicants note that FLP-mediation is not a 100% efficient process and some yeast strains transformed with such 2 $\mu$ m disintegration plasmids may contain a mixture of plasmids, with some containing bacterial drug resistance marker sequences and others without, which is also shown in Figure 9 and at Col. 9, lines 41-47.

Merely for the purpose of clarification applicants' definition of the term "yeast integration plasmid" at p. 13 lines 9-12, p. 29, lines 4-19, pp. 29-30, bridging lines 31-5, and page 29, lines 4-19 of the specification requires that any yeast origin of DNA replication must be removed from the yeast integration plasmids after a necessary amplification step in a yeast host so that the plasmids cannot autonomously replicate in the host yeast strain that is to be genetically modified.

The relevance of applicants' integration plasmid definition is that nowhere in Hinchliffe et al. is it ever taught or suggested that the 2 $\mu$ m sequence must be deleted from the plasmid to allow the plasmid to integrate into a host's genome or, that it is at all desirable even to integrate into the host genome. Hence, the teachings of Hinchliffe et al. only relate to 2 $\mu$ m-derived vectors which autonomously replicate in yeast. After the FLP-mediated recombination has taken place, the Hinchliffe et al. plasmids still contain the 2 $\mu$ m origin of replication which means that those plasmids do not integrate into the host genome ever.

Further, because the Hinchliffe et al. 2 $\mu$ m plasmids do not integrate into the host genome and only exist episomally,



they do not contain any gene (i.e., LEU2, URA3, TRP1) that can function as a targeting gene marker to insert the gene of interest into the yeast genome. Thus, it is not the case that genes of interest could ever be inserted into a host genome and nowhere does Hinchliffe et al. approach the subject of the desirability of inserting genes of interest into a host genome. By contrast, applicants' claims require genes of interest to be inserted into target gene mutation loci in the host yeast's genome.

Hoffman et al.'s teaching of co-expression in diploid yeast of two genes of interest from separate plasmids bearing 2µm origins of replication continues to add nothing to the teachings of Hinchliffe et al. that would render the claimed inventions obvious. Again, the use of 2µm plasmids by Hoffman et al. means that there is no possible way that genes of interest can be inserted and integrated into a host yeast's genome. Hoffman et al.'s use of multiple gene markers such as LEU2d and TRP1/URA3 has no relationship to the use of these or similar gene markers by the applicants, bearing in mind a method is not being claimed. Hinchliffe et al.'s gene markers are only used for selection and maintenance of the episomal plasmids in yeast and can never be used for targeting a host genome because Hinchliffe et al.'s plasmids are extrachromosomal, 2µm-based and therefore never integrate into a host yeast genome. In sum, these are simply gene markers on 2µm plasmids that are independent of cell or chromosomal division.

Hoffman et al. also teaches nothing about inserting multiple copies of a gene in a haploid yeast as recited in the claims.

The Examiner further stated in the first Office

Action:

While neither of the two markers on a plasmid is used for targeting (integration), this limitation in Applicants' claims is merely a recitation of intended use, and the structure of the markers on Hoffman et al.'s plasmids could function in this way, and thus the recitation of intended use for either marker does not confer patentability . . .

(Emphasis added.)

Applicants maintain it is relevant to say again that Hoffman et al.'s markers are only used for selection and maintenance in yeast and can never be used for targeting because Hoffman's et al.'s 2 $\mu$ m-based plasmids are extrachromosomal and never integrate into a host yeast's genome. So long as the plasmids maintain a 2 $\mu$ m sequence, this will always be the case. It is impossible for Hoffman et al.'s gene markers to function in the same way that applicants's LEU2 or TRP1/URA3 do because these genes are carried on the wrong kind of plasmid if the goal is to integrate one or more genes of interest into a host genome, and integration is the goal in this case. One can never arrive at the cells of the pending claims by looking to the teachings of Hoffman, et al. who rely exclusively on the use of 2 $\mu$ m plasmids.

The remainder of the Examiner's remarks about Hoffman et al.'s teachings in light of them not using integrative plasmids regarding various S. cerevisiae haploid strains, auxotrophies, mating types, and mating strains to form a diploid containing two plasmids which express two different genes are respectfully believed to be irrelevant. Hoffman et al. provides no teachings regarding any way to integrate copies of a gene of interest into a host yeast genome. That Hoffman et al. do not teach plasmids without drug resistance genes is simply an additional point of irrelevance.

The Buxton reference similarly adds nothing to the teachings of Hinchliffe et al. and Hoffman et al. to render the claimed invention obvious

Buxton's teachings of the desirability of expressing foreign genes in yeast with vectors that avoid the use of bacterial DNA, and its teachings of making plasmids to that end are nonetheless irrelevant because Buxton only teaches plasmids that contain the 2 $\mu$ m sequences, meaning the plasmids are episomal and not integrative and thus Buxton's plasmids can never be used to make yeast cells with genes of interest stably integrated into the genomes thereof as required by the instant claims. Buxton neither teaches nor suggests anything about how to make yeast integration plasmids that do not contain bacterial DNA.

As to Buxton's teachings about avoiding bacterial DNA, the first method of which the Examiner says is similar to that of Hinchliffe, et al., such teachings are similarly lacking in

relevance in light of the requirement in applicants's claims that genes of interest be integrated into the host genome. The Buxton plasmids contain bacterial fragments until the yeast recombination occurs, but more importantly, such plasmids do not integrate due to the presence of the 2 $\mu$ m element (See, Figs. 1A and 1B where "ORI" represents the S. cerevisiae origin of replication which indicates the plasmid is 2 $\mu$ m ). The second way Buxton prepares plasmids, described at Col. 5, lines 50-60, uses a eukaryotic host, preferably yeast, to carry out the plasmid DNA recombination to excise the bacterial DNA. However the resulting plasmid still contains the 2 $\mu$ m sequence and is therefore not one that can integrate either. The use of yeast genes such as URA3 and LEU2 are only used by Buxton as traditional yeast selection yeast markers and remain inexorably extrachromosomal, and concomitantly nonintegrated, as discussed above for Hoffman, et al. These elements, because they are carried on 2 $\mu$ m plasmids, do not lead to the cells of the yeast.

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